REPORT

Mutations in *CSPP1*, Encoding a Core Centrosomal Protein, Cause a Range of Ciliopathy Phenotypes in Humans

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Ciliopathies are characterized by a pattern of multisystem involvement that is consistent with the developmental role of the primary cilium. Within this biological module, mutations in genes that encode components of the cilium and its anchoring structure, the basal body, are the major contributors to both disease causality and modification. However, despite rapid advances in this field, the majority of the genes that drive ciliopathies and the mechanisms that govern the pronounced phenotypic variability of this group of disorders remain poorly understood. Here, we show that mutations in *CSPP1*, which encodes a core centrosomal protein, are disease causing on the basis of the independent identification of two homozygous truncating mutations in three consanguineous families (one Arab and two Hutterite) affected by variable ciliopathy phenotypes ranging from Joubert syndrome to the more severe Meckel-Gruber syndrome with perinatal lethality and occipital encephalocele. Consistent with the recently described role of CSPP1 in ciliogenesis, we show that mutant fibroblasts from one affected individual have severely impaired ciliogenesis with concomitant defects in sonic hedgehog (SHH) signaling. Our results expand the list of centrosomal proteins implicated in human ciliopathies.

The centrosome is a nonmembranous cellular organelle composed of a pair of unequal centrioles embedded in an electron-dense matrix known as the pericentriolar matrix.[1] Centrosomes are best known for their role during mitosis, when they undergo duplication and migrate from their perinuclear location to opposite poles in the cell, where they organize the microtubules that are bundled as spindles. These spindles emanate from each centrosome and tether the chromosomes by their kinetochores, an essential requirement for normal alignment during metaphase and proper segregation during anaphase.[2] However, centrosomes also play important regulatory roles for microtubules in nonmitotic cells; these include regulation of intracellular transport[3] and formation of the primary cilium through a series of highly orchestrated steps involving the relocation of the centrosome to the cell surface and subsequent recruitment of key structural and functional proteins.[4] The primary cilium can function as a mechanosensor, but its role in signal transduction during development is increasingly recognized in view of the widespread developmental anomalies that accompany primary-cilium defects, collectively known as ciliopathies.[5,6]

Ciliopathies comprise a wide-range of phenotypes, consistent with the near ubiquitous presence of primary cilia in mammalian tissues. Over 60 genes have been associated with various ciliopathy phenotypes, and it has become increasingly clear with the plethora of reports that link apparently distinct clinical phenotypes to mutations in the same individual genes that these phenotypes represent a spectrum in which isolated retinal dystrophy or nephronophthisis (MIM 256100) represent the mild end and perinatal lethal Meckel-Gruber syndrome (MKS [MIM 249000]) with severe brain malformation, polydactyly, and multicystic and/or dysplastic kidneys represents the extreme end.[7–9] The subset of affected individuals who can be explained by mutations in recognized genes varies greatly. For example, primary driver mutations have been identified in the majority of individuals with the intermediate Bardet-Biedl ciliopathy (MIM 209900),[10] whereas for other ciliopathies, such as Joubert syndrome (JBTS [MIM 213300]) and MKS, additional genes remain to be identified.[11,12] In this report, we describe three families affected by JBTS or a syndrome similar to MKS (Table 1) but not linked to any of the established genes and identify *CSPP1* (MIM 611654) as another member of the genes associated with ciliopathy phenotypes in humans.

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Table 1. Clinical Features of Each of the Affected Individuals in This Study

	Family 1		Family 2	Family 3	
Characteristics	**V:1**	**V:3**	**II:1**	**II:1**	**II:2**
Age	stillbirth (26 weeks)	stillbirth (18 weeks)	7 years	28 days (died of unknown cause)	24 days (died of unknown cause)
Gender	male	female	female	female	female
Ethnicity	Saudi	Saudi	Hutterite	Hutterite	Hutterite
Consanguinity	yes	yes	yes	yes	yes
CNS	hydranencephaly, occipital encephalocele	occipital encephalocele	molar tooth sign, heterotopia	molar tooth sign, posterior fossa cyst	molar tooth sign, posterior fossa cyst
Kidneys	hyperechogenic	hyperechogenic	normal	normal imaging	normal imaging
Craniofacial features	single nostril	single nostril	normal	normal	normal
Eyes	anophthalmia	partially fused eyes	strabismus	not available	not available
Others	-	-	tracheesophageal fistula (also in sister unaffected with CNS malformations and not homozygous for CSPP1)	-	-

The first family (family 1) is from Saudi Arabia and consists of healthy parents who are first cousins once removed (Table 1 and Figure 1A). Their first pregnancy was induced at 26 weeks when prenatal sonography showed hydranencephaly, a single nostril, and bilateral hyperechogenic kidneys. The baby was a stillborn male, delivered vaginally after cephalocentesis, with a weight of 1,990 g and occipitofrontal circumference (OFC) of 39 cm. He had large fontanels and wide cranial sutures, occipital encephalocele, anophthalmia, and a single nostril (Figures 1B and 1C). The second pregnancy ended in a spontaneous first-trimester abortion. The third pregnancy was similar to the first in that it showed sonographic findings of occipital encephalocele and bilateral hyperechogenic kidneys (Figures 1D–1F). She was stillborn at 18 weeks and was found to have, in addition to encephalocele, partially fused eyes.

The second family (family 2) is a Schmiedeleut Hutterite Canadian family with one child affected by typical clinical and radiological features of JBTS (Table 1 and Figures 1A, 1G, and 1H). The proband is now 7 years old and has a history of global developmental delay, hypotonia, ataxia, and strabismus. Neuroimaging confirmed the presence of a molar tooth sign. She is otherwise healthy, with the exception of a history of tracheoesophageal fistula, also seen in her otherwise healthy sister, who has age-appropriate development and normal neuroimaging.

The third family (family 3) is also a Schmiedeleut Hutterite Canadian family with two children affected by classic JBTS (Table 1 and Figure 1A). The first child was born at 42 weeks and received care in the neonatal intensive care unit for respiratory distress. At birth, her weight was 3,979 g and her OFC was 37.5 cm. Brain MRI showed absent inferior cerebellar vermis, dysplastic superior cerebellar vermis, posterior fossa cyst communicating with the fourth ventricle, and a molar tooth sign secondary to thickened horizontal superior cerebellar peduncles and a reduced anteroposterior (AP) dimension of the mesencephalon. She died at 28 days. In the second child, a Dandy-Walker variant was noted antenatally at the age of 19 weeks, suggesting a recurrence. Brain MRI showed that the AP dimension of the mesencephalon was smaller than expected. The superior cerebellar peduncles were thick and had a more parallel configuration, giving rise to the molar tooth sign. The cerebellar vermis appeared hypoplastic, the medulla was mildly narrowed, and a mega cisterna magna was also seen. This child died at 3 weeks of age. Autopsy was not performed on either child, but neither had clinical evidence of renal malformations or other congenital anomalies. The Hutterite founder mutation in *TMEM237* (MIM 614423)[14] and the recurrent *NPHP1* (MIM 607100) deletion, which is common in the Hutterites[13] and shown rarely to cause JBTS,[15] were excluded in the proband from each Hutterite family prior to further investigations.

Family 1 was recruited according to a protocol approved by the institutional review board (IRB) at King Faisal Specialist Hospital and Research Center, whereas families 2 and 3 were recruited according to a protocol approved by the University of Calgary IRB; written informed consent was obtained from all research participants. This was followed by DNA extraction from whole blood and autozygome determination as previously described.[16–18] None of these families mapped to any of the genes associated with JBTS or MKS. Therefore, the probands from families 1 and 2 were exome sequenced, and the resulting variants were filtered by consideration of only homozygous coding

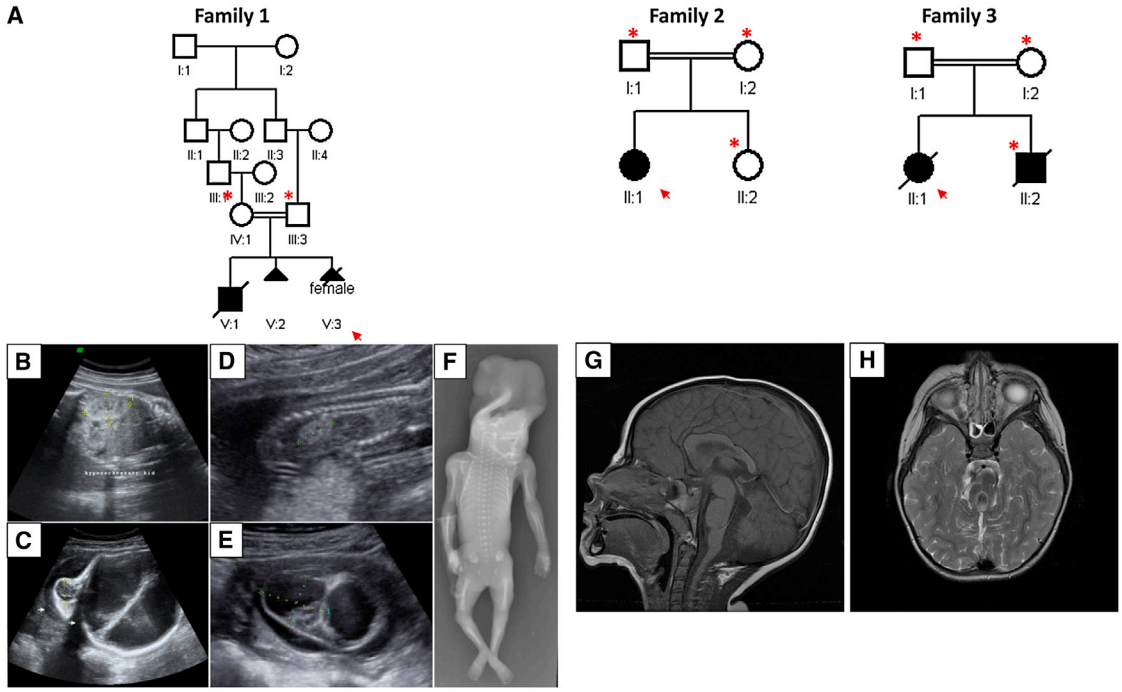

Figure 1. Identification of Ciliopathy Phenotypes in Three Consanguineous Families
(A) Pedigrees of the three study families are shown. The proband is indicated in each pedigree by an arrow, and asterisks denote individuals whose DNA was available for analysis. Please note that the degree of consanguinity between I:1 and I:2 in families 2 and 3 is uncertain but that the average relationship is first cousins once removed.[13]
(B and C) Prenatal sonographic images of V:1 in family 1 show echogenic kidneys (B) and occipital encephalocele (C).
(D and E) Prenatal sonographic images of V:3 in family 1 show echogenic kidneys (D) and occipital encephalocele (E).
(F) A skeletal survey of V:3 in family 1 shows a severe skull defect.
(G and H) A side view (G) and top view (H) of MRI of the family 2 proband show typical cerebellar involvement of JBTS.

and/or splicing variants that were absent from publically available databases and that occurred within the autozygome of each proband (Table S1, available online).[17,19] Consistent with the nature of their founder population, exome analysis of Hutterite family 2 highlighted only a single variant in *CSPP1*, c.363_364delTA (RefSeq accession number NM_024790.6), which is predicted to cause premature truncation of the protein (p.His121Glnfs*22). This mutation was subsequently identified by Sanger sequencing in Hutterite family 3 (Figure 2). In family 1, analysis of the homozygous exome variants restricted to the autozygome revealed one missense variant in *TTC7A* (MIM 609332) and one *CSPP1* frameshift deletion (c.2244_2247del [RefSeq NM_024790.6]) that is also predicted to cause premature truncation (p.Glu750Lysfs*7; Figure 2). *TTC7A* is unlikely to play a causal role in family 1 given its recent link to a Mendelian form of immunodeficiency and intestinal atresia, a phenotype that does not overlap with MKS or JBTS.[20,21] Homozygosity for the *CSPP1* mutation segregated with the affected status of the children in each of the three families. Given the established overlap in the genetic etiology of JBTS and MKS, it is likely that the convergence of exome variant filtration in these families on homozygous truncating variants in *CSPP1* is explained by the causal nature of these variants. In addition, *CSPP1* has previously been proposed as a candidate ciliopathy gene given its role in ciliary biology.[22]

CSPP1 encodes two protein isoforms: CSPP1 and CSPP-L, in which the "L" denotes the longer sequence that is derived by an extended N terminus (an additional 294 amino acids) and an interstitial insertion of 51 amino acids between the glutamine-rich coiled-coiled domains (Figure 2). It was first identified in a screen for genes that are overexpressed in B cell lymphoma and was found later to encode a core centrosomal protein whose deficiency leads to delayed cell-cycle progression.[23] Although the exact mechanism remains unclear, CSPP1 was found to localize in the central spindles during metaphase and the central furrow during anaphase and cytokinesis.[24] The nonmitotic function of CSPP1 was demonstrated recently when it was shown that CSPP1 localizes to the transition zone, as well as to the axoneme of the primary cilium, in a pattern identical to that of the products of other genes associated with ciliopathy phenotypes; such genes include *NPHP1*, *NPHP4* (MIM 607215), and *RPGRIP1L* (*NPHP8* [MIM 610937]).[22] Furthermore, RPGRIP1L (NPHP8) can interact directly with CSPP1, and loss of this interaction leads to mislocalization of RPGRIP1L.[22] Moreover, knockdown of *CSPP1* results in a severe ciliogenesis defect.[22] In view of the above, and given that all genes associated with JBTS and MKS have

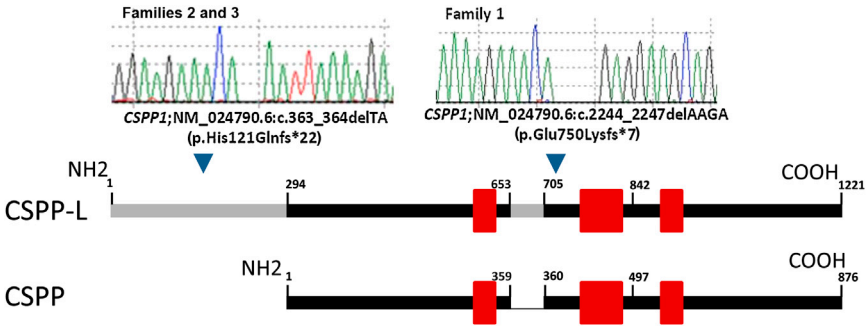

Figure 2. Identification of Two Homozygous Truncating Mutations in *CSPP1*
Schematic of the CSPP and CSPP-L isoforms. The locations of the two truncating CSPP1 alterations identified in the three families are indicated along with the *CSPP1* sequence chromatograms. Red boxes indicate potential coiled-coil regions.

been found to play a role in ciliary biology,[9] we asked whether the truncating variants we identified in *CSPP1* similarly impair ciliogenesis and/or ciliary function. With informed consent, a skin biopsy was obtained from the proband in family 1. Using a standard serum-starvation-induced ciliogenesis assay,[25] we observed that the frequency of ciliated fibroblasts from the proband was markedly lower than that of control fibroblasts. Furthermore, by assessing the mRNA expression of *GLI1* (MIM 165220) in the affected individual's fibroblasts after SAG stimulation and serum starvation, we found that sonic hedgehog (SHH) signaling was markedly impaired, indicating that the ciliogenesis defect we observed has downstream consequences (Figure 3). No fibroblasts were available for testing the effect of the Hutterite mutation on ciliogenesis, but we suspect that it would result in a similar defect because it has been shown that knockdown of the transcript that encodes CSPP-L, despite sparing CSPP1, is sufficient to cause a ciliogenesis defect.[22] To assess the effect of the *CSPP1* mutation on the cell cycle, we used flow cytometry to perform cell-cycle analysis in the fibroblasts from the proband in family 1. We observed no significant difference in the G0/G1 and G2/M cell-cycle phases between this affected individual's cells and control cells (Figure S1).

Our findings are consistent with what is known about the two isoforms of CSPP1 and allow us to speculate on a potential genotype-phenotype correlation. We posit that the redundancy between the two isoforms in certain ciliary functions might contribute, at least in part, to the differences between the clinical presentations of the Hutterite and Saudi mutations. The JBTS phenotype observed with the Hutterite mutation (families 2 and 3) presumably spares the short isoform, whereas the MKS phenotype caused by the Saudi mutation (family 1) truncates both isoforms. Both isoforms share similar expression profiles (expression in the brain, eyes, and kidneys) in the developing mouse embryo and both colocalize, interact, and stabilize RPGRIP1L in the transition zone.[22] Indeed, we show that the ciliary localization of RPGRIP1L was completely lost in the fibroblasts from the proband in family 1 (Figure 4). We note, however, that the clinical variability even within the Hutterite families ranged from neonatal death to relatively milder involvement in an older child. Therefore, any potential role for the two CSPP1 isoforms in affecting clinical severity would be

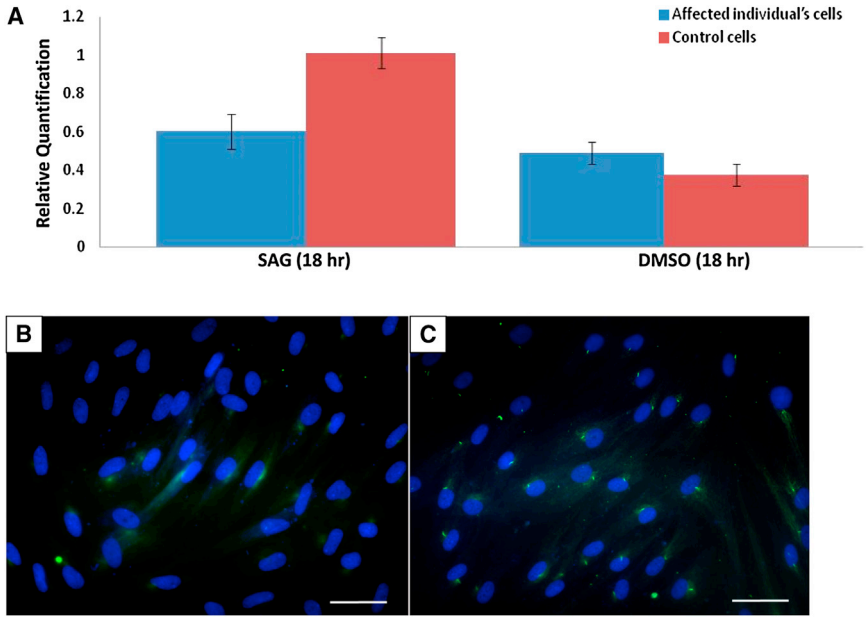

Figure 3. CSPP1-Related Ciliopathy Is Associated with Impaired SHH Signaling and Ciliogenesis Defects
(A) mRNA expression of *GLI1* was markedly lower in fibroblasts from V:3 in family 1 than in control fibroblasts in response to SAG stimulation of 100 nM for 18 hr ($p < 0.0084$ on the basis of three independent experiments each performed in triplicate). Error bars represent the SEM.
(B and C) Immunofluorescence images of serum-starved fibroblasts from the affected individual in family 3 (B) and control fibroblasts (C) stained for the ciliary marker acetylated α-tubulin (Sigma-Aldrich) (green) and DNA (blue). Compared to controls, fibroblasts from V:3 in family 1 showed a marked ciliogenesis defect at 40× magnification. Scale bars represent 50 μm.

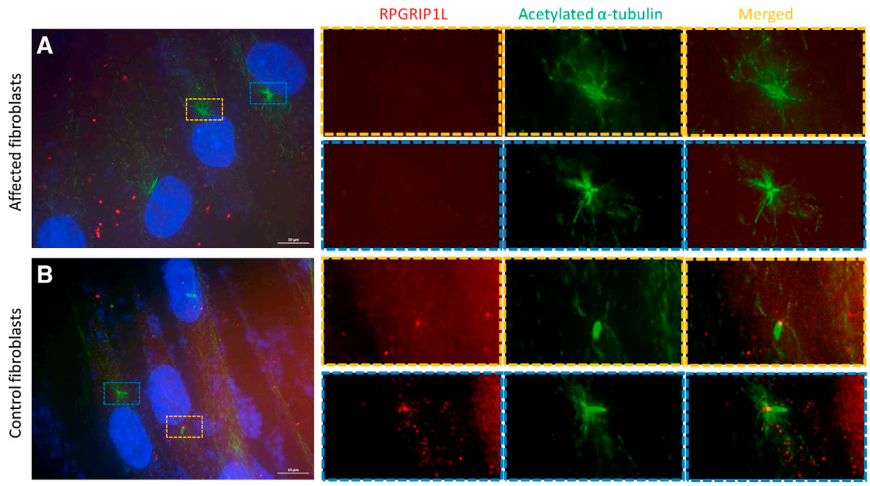

Figure 4. Abnormal Localization of RPGRIP1L in Cells with a *CSPP1* Mutation
Immunofluorescence images of serum-starved fibroblasts from the affected individual in family 3 (A) and control fibroblasts (B) stained for the ciliary marker acetylated α-tubulin (green), RPGRIP1L (Proteintech, catalog no. 55160-1-AP) (red), and DNA (blue). (A) The localization of RPGRIP1L at the basal body was lost in mutant fibroblasts. (B) RPGRIP1L localized normally at the basal body of the cilia in control fibroblasts.

unlikely to occur in isolation, and additional factors are predicted to be involved. To investigate the contribution of *CSPP1* to MKS cases, we sequenced this gene in 89 affected individuals by using a PCR amplicon high-throughput sequencing strategy on an Ion Torrent platform according to the manufacturer's (Life Technologies) instructions and by using subsequent Sanger-based confirmatory sequencing of all alleles found at <1% minor allele frequency in the National Heart, Lung, and Blood Institute (NHLBI) Exome Sequencing Project Exome Variant Server (EVS). We did not identify any truncating mutations in this cohort. We did find three missense variants of unknown significance (c.2219G>A [p.Arg740His], c.2966G>A [p.Arg989Gln], and c.1376 C>G [p.Ser459Cys]), the first two of which were carried by individuals in a heterozygous state and the third of which was carried in a homozygous state. These variants are absent in variant databases (1000 Genomes and the NHLBI EVS) and are predicted to be pathogenic by PolyPhen-2, SIFT, and MutationTaster. The homozygous variant was carried by an individual identified previously to also harbor two variants (one truncating and one missense) in *RPGRIP1L*, mutations in which are known to cause JBTS and MKS. Although we are unable to resolve the significance of these alleles, it is possible that *CSPP1* is the primary driver instead of *RPGRIP1L*. Thus, it appears that *CSPP1* mutations account for only a small percentage of MKS alleles.

Germline mutations in genes that encode various centrosomal proteins appear to fall broadly under two categories: (1) microcephaly and/or primordial dwarfism and (2) classical ciliopathy. It appears that the former is the outcome of more severe mitotic dysfunction of the centrosome, whereas the latter is the result of dominating postmitotic ciliary dysfunction. This model incorporates accumulating evidence that the genes associated with either class of disorders encode proteins that are essential for both mitotic and ciliary functions of the centrosome. For example, deficiency of *POC1A* (MIM 614783) and *CENPJ* (MIM 609279) has been shown to impair both mitosis and ciliogenesis, yet mutations in these genes cause primordial dwarfism lacking all of the phenotypic features of a classic ciliopathy.[26–28] Similarly, *CEP290* (MIM 610142), mutations in which result in a wide-array of ciliopathy phenotypes, is known to be essential for mitosis.[29,30] We demonstrate here that mutations in *CSPP1*, another gene with an established function in both mitosis and ciliogenesis, result in a restricted cilia-related phenotype, consistent with our findings of a severe ciliogenesis defect but lack of a significant observable effect on mitosis.

In summary, we identified *CSPP1* truncating mutations in several individuals with ciliopathy phenotypes ranging from JBTS to MKS-like syndromes and observed a ciliogenesis defect in cells from an affected individual. Our findings further delineate the genetic heterogeneity of these two disorders in the Saudi and Hutterite (and potentially other) populations. It remains to be seen whether additional mutations in this gene can result in other ciliopathy phenotypes and whether differential involvement of the two isoforms can indeed provide insight into potential genotype-phenotype correlations.

Supplemental Data

Supplemental Data include one figure and one table and can be found with this article online at http://www.cell.com/AJHG.

Acknowledgments

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Web Resources

The URLs for data presented herein are as follows:

Burrows-Wheeler Aligner, http://bio-bwa.sourceforge.net/
Ensembl Genome Browser, http://www.ensembl.org/index.html
MutationTaster, www.mutationtaster.org/
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org
PolyPhen-2, www.genetics.bwh.harvard.edu/pph2/
SIFT, http://sift.bii.a-star.edu.sg/
UCSC Genome Browser, http://genome.ucsc.edu/

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